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Short Communication

Molecular Characterization of Methicillin-Resistant *Staphylococcus aureus* Clones from a Teaching Hospital in Tehran

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SUMMARY: A total of 52 methicillin-resistant *Staphylococcus aureus* (MRSA) clinical isolates were collected from patients attending the teaching hospital of Tehran University of Medical Sciences. Disks containing antibiotics were used to determine the susceptibility of MRSA isolates. Analysis of *Sma*I macrorestriction profiles of the 52 MRSA isolates were grouped into three PFGE types. The majority of isolates (n = 49) were clustered into only one major PFGE type, designated as pulsotype A; these belonged to SCC*mec* type III or IIIA and showed resistance to ampicillin, ciprofloxacin, cotrimoxazole, erythromycin, gentamicin, and tetracycline. The remaining isolates fell into pulsotypes B and C, both belonging to SCC*mec*-type IV. All MRSA isolates were susceptible to vancomycin, teicoplanin, quinupristin-dalfopristin, linezolid, and tigecycline. The present study shows that a MRSA clone similar to the Brazilian clone (ST 239) of MRSA, which is a multiresistant MRSA clone with a high level of methicillin resistance, is very common in this teaching hospital in Tehran.

Different clones of methicillin-resistant *Staphylococcus aureus* (MRSA) have appeared in different countries (1,2). The Brazilian epidemic clone was first described to be widespread in Brazilian hospitals in 1995 (3). This clone was also found to be disseminated in South America and Europe (4). Other epidemic MRSA clones (EMRSA) such as Iberian, New York/Japan, Pediatric, Berlin, EMRSA-15, and EMRSA-16 have also been identified (5-8). Pulsed-field gel electrophoresis (PFGE) has been reported as the "gold standard" for studying the molecular epidemiology of infections with MRSA (6,7). This paper reports the first study on the common MRSA clone as determined by PFGE in Iran, where MRSA has recently emerged as important pathogen (1,9).

A total of 52 MRSA clinical isolates were cultured from patients attending the teaching hospital of Tehran University of Medical Sciences between January 2006 and March 2007. Only one isolate per patient was included. Isolates were identified at the species level using standard biochemical methods (10). To detect the MRSA strains, the susceptibility of isolates to oxacillin was assessed using the disk diffusion and agar dilution methods. All strains with an oxacillin (methicillin)-resistant phenotype were evaluated for the *mecA* gene by PCR, using a set of previously designed primers (9). Disks containing antibiotics (Mast, Merseyside, UK) were used to determine the susceptibility of MRSA isolates according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (11). *S. aureus* ATCC 29213 was used as a control. The primers used to detect SCC*mec* types have been described by Oliveira and Lencastre (12). Whole genomic DNA was prepared as described by Murchan et al. (6). After digestion with S*ma*I endonuclease, DNAs were separated by field inversion gel electrophoresis (FIGE; Faculty of Engineering, University of Tehran) and the CHEF DR II electrophoresis system (Bio-Rad, Hercules, Calif., USA) for 23 h at 14°C, with an electric field of 6 V/cm in 0.5× TBE buffer. The pulse time was increased from 1 to 30 s. The gels were stained with ethidium bromide (1 μg/ml) and visualized by UV illumination. DNA from *S. aureus* NCTC8325 was prepared in the same way and run as a molecular size standard. The MRSA isolate HU25, representing the Brazilian clone, was included for comparison (3).

All MRSA isolates were susceptible to vancomycin, teicoplanin, quinupristin-dalfopristin, linezolid, and tigecycline, consistent with reports from other countries (13-15).

PFGE analysis of 52 MRSA isolates by FIGE produced three distinct pulsotypes designated as pulsotypes A-C (Fig. 1A). The majority of isolates (n = 49) were clustered into pulsotype A. Analysis by the CHEF DR II apparatus revealed that pulsotype A contained two isolates that differed slightly from pulsotype A (pulsotypes A1 and A2, Table 1). All isolates in pulsotype A belonged to SCC*mec* type III or IIIA. The majority of SCC*mec* type III and IIIA isolates were resistant to ampicillin, ciprofloxacin, cotrimoxazole, erythromycin, gentamicin, and tetracycline. In this study, SCC*mec* typing correlated well with major antimicrobial susceptibility patterns.

The PFGE pattern and antibiogram of nine strains with SCC*mec* type IIIA were identical to strains with SCC*mec* type III. Therefore, they might have originated from the same ancestor, probably through the deletion of a portion including pT181 (12). Based on the visual comparison of banding patterns, the similarity of this clone with the Brazilian/Hungarian clone found in India, Taiwan, China, and Georgia (13-15) was >80%.

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Table 1. Properties of MRSA strains isolated from a teaching hospital in Tehran

No. of isolates	Source	Antimicrobial resistance pattern	SCC*mec* type	PFGE type
19	Wound	AP, CIP, TS, E, GEN, T	III	A
2	Wound	AP, CIP, TS, E, GEN, T, RP	III	A
2	Wound	AP, CIP, TS, E, GEN, T	IIIA	A
5	Blood	AP, CIP, TS, E, GEN, T	III	A
3	Blood	AP, CIP, TS, E, GEN, T	IIIA	A
1	Respiratory secretion	AP, CIP, TS, E, GEN, T	III	A
2	Respiratory secretion	AP, CIP, TS, E, GEN, T	IIIA	A
4	Catheter	AP, CIP, TS, E, GEN, T	III	A
2	Pleural fluid	AP, CIP, TS, E, GEN, T	III	A
2	Synovial fluid	AP, CIP, TS, E, GEN, T	III	A
2	Eye exudate	AP, CIP, TS, E, GEN, T	III	A
2	Eye exudate	AP, CIP, TS, E, GEN, T	IIIA	A
1	Femoral plaque	AP, CIP, TS, E, GEN, T	III	A
1	Wound	AP, CIP, TS, E, GEN, T, C	III	A1
1	Catheter	AP, CIP, TS, E, GEN, T	III	A2
2	Wound	AP, T	IV	B
1	Blood	AP, C, TS	IV	C

AP, ampicillin; CIP, ciprofloxacin; C, chloramphenicol; TS, cotrimoxazole; E, erythromycin; GEN, gentamicin; T, tetracycline; RP, rifampin.

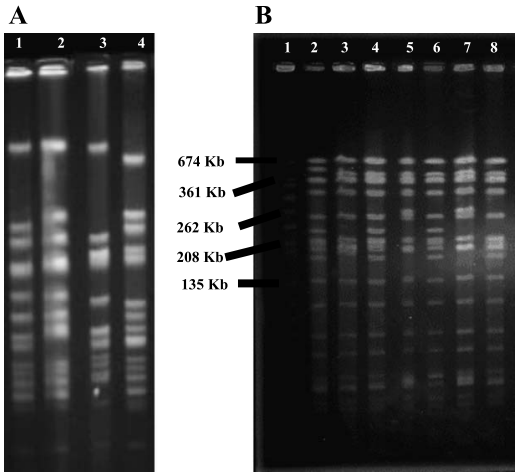

Fig. 1. Pulsed-field gel electrophoresis (PFGE) of *Sma*I macrorestriction fragments of MRSA strains isolated from a teaching hospital in Tehran. (A) Lane 1, *Staphylococcus aureus* NCTC 8325; Lanes 2-4, MRSA clinical isolates (pulsotype C, B, and A, respectively). (B) Lane 1, *Staphylococcus aureus* NCTC 8325; lane 8, HU25 isolate belonging to the Brazilian clone; lanes 2-7, MRSA clinical isolates with SCC*mec* type III and IIIA (pulsotype A) from wound, blood, respiratory secretion, catheter, pleural fluid, and eye exudates, respectively.

Three isolates, belonging to SCC*mec* type IV, fell into pulsotypes B and C. Of the three isolates with SCC*mec* type IV, two were isolated from the wounds of nonhospitalized patients (pulsotype B). The third strain, pulsotype C, was isolated from the blood of a hospitalized patient. Based on the differences in the number of restriction fragments, these isolates showed differences in up to six DNA bands from pulsotype A.

Previous studies have shown that SCC*mec* type IV status is a characteristic of community-acquired (CA)-MRSA strains, which are generally susceptible to non-β-lactam antibiotics and are typically more susceptible to antibiotics than hospital-acquired (HA)-MRSA strains (16,17). However, our three SCC*mec* type IV, isolates were resistant to other antibiotics. This finding is in accordance with those of other studies suggesting that SCC*mec* type IV strains can acquire resistance to non-β-lactam antibiotics in order to survive in the hospital environment (2) or through exposure to antibiotics (18).

In conclusion, the present study shows that the Brazilian clone of MRSA, which is a multi-resistant MRSA clone with a high level of methicillin resistance, is very common in this teaching hospital in Tehran. We do not know the representation of this clone in other settings. Although there is one report on the molecular epidemiology of MRSA in Iran using the PCR method (19), this is the first published study using PFGE in Iran.

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